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Mabel Grace Morris

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The *Butler University Botanical Studies* journal was published by the Botany Department of Butler University, Indianapolis, Indiana, from 1929 to 1964. The scientific journal featured original papers primarily on plant ecology, taxonomy, and microbiology. The papers contain valuable historical studies, especially floristic surveys that document Indiana's vegetation in past decades. Authors were Butler faculty, current and former master's degree students and undergraduates, and other Indiana botanists. The journal was started by Stanley Cain, noted conservation biologist, and edited through most of its years of production by Ray C. Friesner, Butler's first botanist and founder of the department in 1919. The journal was distributed to learned societies and libraries through exchange.

During the years of the journal's publication, the Butler University Botany Department had an active program of research and student training. 201 bachelor's degrees and 75 master's degrees in Botany were conferred during this period. Thirty-five of these graduates went on to earn doctorates at other institutions.

The Botany Department attracted many notable faculty members and students. Distinguished faculty, in addition to Cain and Friesner , included John E. Potzger, a forest ecologist and palynologist, Willard Nelson Clute, co-founder of the American Fern Society, Marion T. Hall, former director of the Morton Arboretum, C. Mervin Palmer, Rex Webster, and John Pelton. Some of the former undergraduate and master's students who made active contributions to the fields of botany and ecology include Dwight. W. Billings, Fay Kenoyer Daily, William A. Daily, Rexford Daudenmire, Francis Hueber, Frank McCormick, Scott McCoy, Robert Petty, Potzger, Helene Starcs, and Theodore Sperry. Cain, Daubenmire, Potzger, and Billings served as Presidents of the Ecological Society of America.

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A STUDY OF BACTERIA FOUND IN THE DISTRIBUTION SYSTEM OF A WATER PLANT

By MABEL GRACE MORRIS

One of the basic bacteriological findings used in helping to determine the quality and safeness of drinking water is the agar plate count. Raw water samples show the kind of water entering the plant and are a fair indicator of the treatment that will be needed. Plate counts of water samples taken at various places in the plant, such as settling basins or point of chlorination, show the effectiveness of the various treatment. Water leaving the plant is termed plant effluent, and results on this water are of primary concern. Its count should be very low, such as 1-8 per ml, if the water bas been successfully treated.

It is also desirable to obtain plate counts on water samples throughout the distribution system, in order to know in what bacterial condition the consumer is actually receiving the water. It might be presumed that if the effluent samples show low counts, these tap samples would likewise show low counts. The writer found, however, that some tap samples in a certain distribution system often produced very high agar plate counts, amounting to hundreds per ml.

The following general observations, concerning these bacteria, were noted:

1. Nutrient agar plates incubated at 37° C. for 24 hours showed such very small colonies that it was questionable whether they were true colonies. However, further incubation showed that they were definite colonies.

2. If the additional incubation happened to he at 20° C., the colonies showed a light orange pigment.

3. There was a tendency for the high counts to be present in taps farther away from the center of the distribution system.

4. There was no consistency as to whether or not there was residual chlorine present in these samples showing high counts.

5. Several particular tap samples were generally responsihle for all the high counts in the distribution system, although the counts on these samples were not consistent from day to day.

Wilson (21), in a study on the bacteriology of water pipes, states that the most efficiently operated water plants have bacteria present

in the water, as many bacteria as are able to survive the dosage of chlorine lethal for coliform organisms. He further states that, although bacteria may be unable to flourish in a certain environment, they are able to survive there in an inactive state for an indefinite period and multiplication will take place rapidly when the environment is more favorable. Charlton (4), in his work on chlorine-resistance of non-sporeforming bacteria in chlorinated water supplies, encountered pigmented, Gram-negative rods which he assigned to Pseudomonas and Flavobacterium. He found that these bacteria possessed a definitely greater chlorine tolerance than intestinal rod forms.

The report of Levine and co-workers (13) shows the types of non-sporeforming organisms surviving chlorination to include members of the genera Flavobacterium, Micrococcus, Pseudomonas and Aerobacter. Likewise, Shannon (17) found a few Gram-negative yellow-pigmented bacteria belonging to the genus Flavobacterium in his work on samples from the distribution system. He encountered these pigmented bacteria chiefly on plates incubated at 20° C. Bender (1), in his study of microorganisms surviving water chlorination, placed these chromogenic organisms producing water-insoluble pigment in the genus Flavobacterium, and those producing water-soluble pigments in the Pseudomonas group.

Deutschlander (7) suggested that "old mains become coated with inorganic and organic deposits, and the bacteria adhering to these encrustations thrive and multiply. In water not sufficiently chlorinated, there is first a decrease in bacteria due to the sterilizing action of chlorine, but as soon as the chlorine has been utilized the bacteria increase. The residual chlorine progressively diminishes in the mains farther away from the pumping station, so that saprophytes are not prevented from multiplying."

PURPOSE OF THE STUDY

Since other workers have encountered similar high bacterial counts in distribution systems, it is evident that this is not entirely a local problem. An extensive and thorough study of the predominating bacteria found in the tap samples should result in showing both favorable and unfavorable conditions for the growth and multiplication of these bacteria. Although most workers, in their studies on bacteria in chlorinated water supplies, have made identification only to genus, additional identification to species would be most desirable. All of this knowledge should be beneficial to both the bacteriologist and the person responsible for the effective treatment of water.

EXPERIMENTAL RESULTS

The following experiments were carried out on the organisms isolated in this study:

1. Effect of incubation time and temperature on growth.
2. Growth on different culture media.
3. Microscopic characters.
4. Selection of satisfactory medium for growth.
5. Relationship of season to number of bacteria.
6. Presence of the organism in other parts of the water system.
7. Relationship of coliforms to the pigmented organism.
8. Starch hydrolysis.
9. Study of central swellings in the rod forms.
10. Pigment production.
11. Length of cell in relation to age and kind of culture medium.
12. Relationship of morphological changes to time.
13. Effect of hydrogen-ion concentration.
14. Effect of soluble starch concentration.
15. Effect of lactose concentration.
16. Nature of the internal granules in the cell.
17. Cell variation.
18. Characterization and identification.

1. Effect of Incubation Time and Temperature on Growth

Tap samples which showed high counts were plated on nutrient agar. Both the temperature and period of incubation were factors affecting growth of the organism as may be seen in the following table of results.

	Temp. °C.	Time hours	Size of colonies	Pigmentation	Number of colonies	Distinctness of colonies
1.	37	24	pin-point	none	medium	indistinct
2.	37	48	pin-point	none	large	distinct
3.	37	72	very small	none	large	distinct
4.	30	24	pin-point	sl. orange	less than (1)	indistinct
5.	30	48	very small	sl. orange	less than (2)	distinet
6.	30	72	very small	orange	less than (3)	distinct
7.	20	24	no colonies visible			
8.	20	48	pin-point	sl. orange	less than (4)	indistinct
9.	20	72	very small	orange	less than (5)	distinct
10.	37	24	followed by			
	20	48	medium	orange	large	distinct

A prominent characteristic of these bacteria is the development of orange pigmentation at incubation temperatures of either 30° C. or 20° C. but none at 37. A longer period of incubation increases the size, distinctness and number of colonies as well as produces more pigmentation at the lower temperatures. Optimum time and temperature for pigmentation is incubation at 37° C. for 24 hours followed by reincubation at 20° C.

2. Reaction of the Bacteria to Differential Culture Media

Colonies were picked from plates of tap samples which showed high bacterial counts. The cultures were purified by repeated plating, and then inoculated onto the various media and incubated.

Medium	Results
Nutrient agar	light orange, filiform, fair growth, smooth, adherent
Gelatin	saucer-shaped, slow liquefaction, orange
Nutrient broth	no growth
Potato	dry, lustreless, fair growth, coral pink to red
Lactose	no gas, acid of pH 5.1 produced
Dextrose	no gas or acid produced
Salicin	no gas or acid produced
Dulcitol	no gas or acid produced
Sucrose	no gas or acid produced
Egg albumin	very slowly digested, slight orange
Loeffler's blood serum	no growth, no hemolysis
Czapek's agar	slimy, light pink, smooth, fair growth
Cellulose	no digestion, orange pigmentation along surface
Cornmeal	slimy, smooth, light pink, fair growth
Lead acetate agar	slimy, smooth, pink, fair growth, no H_2S
Glycerin asparagine agar	no growth
Litmus milk	no change
Methyl red	no acid produced
Voges-Proskauer	no acetyl methyl carbinol
Nitrate reduction	Nitrates not reduced
Brilliant green bile	no gas
Tyrosinase reaction	no darkening of medium
Rubber	no growth
Hemp	scant growth
Paraffin	no growth
Deep agar	aerobic, growth along surface and upper layer
Nutrient agar with 0.2% soluble starch	orange-pink, very slimy, spreading, abundant growth
Nutrient broth with 0.2% soluble starch	Slight turbidity, moderate sediment

Since all of the pure cultures showed identical results on differential culture media, it is likely that only one species is responsible for all these high counts on certain tap samples.

3. Microscopic Characters of the Organism

Observations were made from various media and after various periods of incubation.

Morphology: filamentous rods which apparently segment into short rods to coccoid forms. Size: filamentous rods 1.2 x 8-60 microns, rods 0.8-1.2 x 2.4-6 microns, coccus 0.3-0.6 microns. Grouping: singly, long or short chains. Gram's stain: negative. Motility: non-motile. Acid-fast: non acid-fast. Colony: hair-like, often granules at outer end, center of colony thicker and denser, rods have swirling effect, deep colonies lens-shaped. Unusual characteristics: *granules,* deeply staining granules distributed throughout longer rods and usually bipolar in short rods, stain well with acetic methylene blue. *Y-forms and globular bodies,* often noted on potato cultures. *Coccoid forms,* more abundant on prolonged incubation, especially abundant on lactose media from which they continue to remain coccoid when transferred to agar slants.

A striking feature of this organism is the pleomorphism, forming filaments, rods and coccus forms. Experiment 11 gives a detailed account of how the morphology is affected by age of culture medium used. On routine microscopic observation of the organism from nutrient agar slant, the growth is so slow that it must be incubated several days, and only normal rods will be observed. Granules are generally not detected unless acetic methylene blue stain is used.

4. Selection of a More Satisfactory Medium than Nutrient Agar

The bacterium was inoculated onto 6 different media and incubated at 37° C. for 24 hours followed by additional incubation at 20° C. The following results were obtained: Nutrient Agar: filiform, smooth, compact, adherent, fair growth. Potato: dry, lustreless, adherent, fair growth. Lead acetate agar: slightly slimy, smooth, spreading, fair growth. Cornmeal agar: slightly slimy, smooth, spreading, fair growth. Czapek's agar: slightly slimy, smooth, spreading, fair growth. Nutrient agar with 0.2% soluble starch: very slimy, mucous growth, colonies spreading and much larger than those on nutrient agar; growth abundant.

Satisfactory inoculations from nutrient agar cultures are very difficult. The nutrient agar to which 0.2% soluble starch has been added shows the best growth characteristics for successful inoculations. The very slimy, spreading and mucous character of the growth on 0.2% soluble starch nutrient agar raises the question as to whether the bacterium has this quality while in the water mains. Sanborn (16), in a study of bacteria which are difficult to eliminate by the use of chlorine treatment, says that the slimy bacteria are able to resist the effects of chemicals. Perhaps this quality of sliminess may explain why this bacterium is often found in tap samples which have residual chlorine.

5. Correlation Between Seasons of the Year and High Bacterial Counts

Samples from 9 taps which often showed high counts were plated twice weekly on nutrient agar plates and incubated at 37° C. for 24 hours. Counts were recorded over a period of 19 months. In the following table "o" indicates low counts and "x" indicates high counts both being based on monthly averages.

	Tap Samples								
	1	2	3	4	5	6	7	8	9
January	o	o	o	o	o	o	x	o	o
February	o	o	o	o	x	o	x	o	x
Mareh	o	o	o	o	o	o	o	o	o
April	o	o	o	o	o	o	o	o	o
May	o	x	o	o	o	o	o	x	o
June	x	x	o	o	x	x	x	x	o
July	x	x	o	x	x	x	x	x	o
August	x	x	x	x	x	x	x	x	o
September	x	x	x	x	x	x	x	x	x
Oetober	x	x	x	x	x	x	x	x	x
November	x	x	x	x	x	x	x	x	x
December	x	x	x	x	x	x	x	x	x
January	x	o	x	o	x	x	x	x	o
February	x	x	x	o	x	x	x	x	o
March	o	o	o	o	o	o	o	o	x
Apríl	o	o	o	o	o	o	o	o	o
May	o	o	o	o	o	o	o	o	o
June	o	x	x	o	x	o	x	x	o
July	x	x	x	x	x	o	x	x	x

These results show that higher counts appear during the summer, autumn and early winter months with lower counts during late winter and early spring months.

6. Presence of Orange-Pigmented Colonies in Other Parts of the Water System

Samples of raw water, plant water, plant effluent and tap samples showing low counts were plated at various time intervals on nutrient agar plates and were incubated at 37° C. for 24 hours and at 20° C. for 48 hours additional incubation. No orange-pigmented colonies were ever observed on the plates of these samples. Evidently, the bacterium is not present in the water before it reaches the distribution system. Neither is it present in all tap samples.

7. Relationship of Coliforms to Pigmented Organisms

Fifty samples of tap water which produced pigmented colonies on nutrient agar plates were tested for coliforms, according to "Standard Methods" for water analysis. Results showed that coliforms were not present in any of the samples tested. This indicates that chlorine may be sufficient for coliforms, yet insufficient for killing these orange-pigmented organisms. This agrees with Charlton (4) who found, in a study on chlorinated water supplies, that pigmented bacteria had a greater chlorine tolerance than coliforms.

8. Starch Hydrolysis

The organisms produced extensive destruction of starch in nutrient agar containing 0.2% soluble starch. There was also complete hydrolysis of starch in potato-starch nutrient broth. The standard Fehling test indicated production of glucose. The organism is, thus, actively diastatic, breaking up starch with rapidity and reducing it to glucose.

9. Study of Central Swellings in Rod Forms

Microscopic examinations were made of bacteria from various culture media in an effort to observe the conditions under which these central swellings occur. The swellings range from an enlarged central portion of the rod to spherical bodies often up to 4 microns in diameter in rods which were only 0.8 micron wide. Few terminal swellings were noted. These globular bodies were observed on both young and old cultures on potato slants. Inoculation on potato slants were not very successful, but whenever growth was obtained some swollen bodies were always observed.

The only other medium upon which they appear is sometimes on nutrient agar which has been inoculated from 0.2% soluble-starch nutrient agar. However, repeated findings show that when inoculations are made onto either nutrient agar or 0.2% soluble-starch agar, the bulbous swellings do not appear, normal rods only being present. Inoculations from potato slants to any other culture medium failed to produce the swellings.

Gillespie and Rettger (8), in their work on variant cells of *Bacillus megatherium,* state: "Variant types such as globular bodies or filaments with bulbous swellings never produced organisms of the same type. In environments responsible for their formation, they simply increase in size up to a certain point and then remain dormant until death and autolysis ensue. When transferred to new and wholesome surroundings, variable variant cells returned to 'normal' rod forms. Cells of unusual shape seem to form in response to unfavorable environmental conditions. They were presumably simple adaptive variants and did not appear to represent stages in an orderly life cycle."

The results of this experiment agree with Gillespie and Rettger in that rod-form bacteria with globular bodies produce "normal" rod forms and in that variant types occur under certain environmental conditions.

10. Pigment Production

The pigment was insoluble at room temperatures in water, alcohol and xylol. The optimum time and temperature for pigmentation was 24 hours at 37° C. followed by re-incubation at 20° C. for an additional 48 hours. The 0.2% soluble-starch agar produced good pigmentation in 24 hours at 37° C.

Pigmentation as affected by medium: organisms were incubated on each of the following media at 37° C. for 24 hours followed by re-incubation at 20° C. for 48 hours (exception being made for gelatin). Results were: on 0.2% soluble-starch nutrient agar, orange-pink pigmentation; on gelatin, orange pigment; on nutrient agar, light orange; on Czapek's agar, pale pink to colorless; on corn-meal agar, pale pink to colorless; on lead acetate agar, pink; on potato, vermillion-red.

*Effect of hydrogen-ion concentration*s using 0.2% soluble-starch nutrient agar the following results were obtained.

Time	pH 6.2	pH 6.6	pH 7.4
48 hrs.	orange-pink	light orange-pink	very light orange-pink
1 wk.	deep orange-pink	orange-pink	orange-pink
2 wks.	almost colorless	light orange-pink	almost colorless

Media with low pH values give greater intensity of pigment, up to 2 weeks incubation. After that time, a medium or circum-neutral reaction shows the best pigmentation.

Pigmentation as related to growth: medium of pH 6.2 incubated for 1 week shows deepest pigment as well as most abundant growth, while medium of pH 7.4 incubated for 48 hours shows lightest pigment and scant growth. Factors contributing toward good growth also increase density of pigment. Most abundant growth was produced on nutrient agar containing 5% soluble starch. Pigmentation was light orange when the nutrient medium contained no soluble starch, orange when it contained 0.2% soluble starch, orange-pink when 1% soluble starch was present, and deepest pink when 5% soluble starch was present. Charlton (4), Shannon (17), Henrici (9) and Bender (1) also observed pigmented bacteria surviving water chlorination. Their studies did not include factors affecting pigmentation.

11. Length of Cell in Relation to Age and Kind of Medium Used

The organism was inoculated onto 10 different media and microscopic examination made at certain time intervals. Longer filamentous rods seem to occur during the growth period while shorter rods appear afterward. Longer rods seem to develop on rich, moist media which are most favorable for growth. Shorter rods develop after longer incubation when the medium is less moist and less favorable to growth. This agrees with the results of Gillespie and Rettger (9) and those of Topley (19). The following table shows results.

Medium	Length in microns 30-60	20-30	10-20	6-10	1-6	coccoid
Nutrient agar						
24 hrs.		x	x	x	x	
72 hrs.		x	x	x	x	
1 wk.			x	x		
6 wks.		x	x	x		
2.5 mo.					x	
0.2% soluble starch nutrient agar						
24 hrs.	x	x	x	x	x	
72 hrs.					x	
1 wk.		x	x	x		
6 wks.				x		
0.2% soluble starch nutrient broth						
1 wk.		x	x	x		
6 wks.				x		x
2.5 mo.					x	x
Potato						
1 wk.				x	x	
6 wks.			x	x	x	
2.5 mo.					x	
Czapek's						
72 hrs.				x		
1 wk.				x		
6 wks.				x		
2.5 mo.					x	
Cellulose						
1 wk.	x	x	x			
6 wks.					x	x
2.5 mo.					x	x
Cornmeal agar						
1 wk.				x	x	
6 wks.					x	
Gelatin						
1 wk.				x	x	x
2.5 mo.						x

Medium	Length in microns 30-60	20-30	10-20	6-10	1-6	coccoid
Nutrient broth						
6 wks.					x	x
2.5 mo.						x
Phenol red lactose						
1 wk.			x	x		
6 wks.					x	x
2.5 mo.						x

12. Relation of Morphological Changes to Time

The bacteria from actively growing cultures were observed microscopically at frequent intervals over a period of three days. The length and width of the rods remained constant during all periods of the first two days while toward the end of the third day the rods slightly decreased in both length and width. No coccus forms were observed. The granules in the rods were clearly observed during all periods of the examination. This bacterium seems to undergo a slow, gradual decrease in size and later, when reduced to a coccoid form, is unable to change back to a rod form, and also loses its power of reproduction. An exception to this is found in the coccus forms from lactose (exp. 17). There is, thus, no indication of a morphological time sycle.

Colien (5) made a 30-hour growth study of a yellow pigment-producing coccus. A change was observed from coccus to rods to filaments, to rods, to coccoid forms, to original form; this morphological cycle being completed in 30 hours. The present results show no evidence of a time cycle such as Colien observed. The results are more nearly similar to Topley's (19) discussion of Actinomyces, He says that the filaments occur in young cultures and later (24 hours to 3 weeks) the filaments segment into rods and coccoid forms.

13. Effect of H-ion Concentration

The bacterium was inoculated on both 0.2% soluble-starch nutrient agar and 0.2% soluble starch nutrient broth of various pH values. Growth and microscopic characters were observed at different time intervals. Results show that the neutral and more acid media produce slightly better growth than those more alkaline. Media with pH 6.6 seem to be the most satisfactory for rods since the organisms remain

as rods over a longer period of time than when grown on media with pH 6.2 or 7.4. On nutrient broth with 0.2% soluble starch, a reaction of pH 5.7 to 6.0 gave best results. The more acid medium produced shorter rods and coccoid forms while the circum-neutral and slightly alkaline media show longest rods. These results are at variance with the findings of Novak and Henrici (14), on a pleomorphic bacterium, wherein H-ion concentration did not affect morphology.

14. Effect of Concentration of Soluble Starch on Growth and Morphology of the Bacterium

The bacterium was inoculated onto nutrient agar with varying concentrations of soluble starch. Growth characteristics and microscopic examination at certain time intervals showed that as the amount of starch was increased (up to 5%) the amount of growth increased. The slimy character of the organism also increases with increased concentration of starch. The length of rods also increases as the concentration of starch is increased. Cultures incubated 2 weeks and then streaked on nutrient agar slants and incubated at 37° C. for 48 hours show that increased concentration of starch is followed by an increase in the amount of growth.

15. Effect of Lactose Concentration

The bacterium was inoculated into nutrient brotb with amounts of lactose varying from 0 through 0.5%, 1%, 5%, to 10%. Best growth as determined by turbidity and sediment, is shown in the nutrient broth with 0.5% lactose. This also shows the longest rods and the rod condition continues for the longest period as compared with other concentrations. This partially agrees with Novak and Henrici (14) who found, in their work showing relationships between staphylococci and actinomycetes, that the inciting substance which caused the morphological transformation from the coccus to the rod form was a sugar.

16. Nature of Internal Granules in the Cells

Since the organisms were unable to grow on soluble-starch nutrient agar containing 0.3% sodium sulphate nor on similar media containing similar concentrations of sodium sulphite it was concluded that the granules are not sulphur. They also gave negative results when tested for starch with iodine.

The stain most satisfactory for microscopic observation of the granules was found to be acetic methylene blue.

The bacterium was inoculated onto 10 different media as follows: nutrient agar, nutrient agar with 0.2% soluble starch, potato, nutrient broth with 0.2% soluble starch, Czapek's agar, cornmeal agar, gelatin, lead acetate agar, and lactose agar. Observations after 24 hours and after one week show that few granules were produced on most media. But on prolonged incubation, rods show more granules and the rods ultimately become more indistinct until only packets of cocci remain. Cocci might be a more stable form produced under less favorable conditions. Coccus forms may not be able to reproduce except under very favorable conditions. Cocci, when reproduced, as from lactose, produce cocci and not rods.

17. Cell Variation

Bacterial rods, on certain media and after certain periods of time, show coccoid bacteria. Often, also, structureless, granular, lightly staining material is observed. Colien (5), in his work on microbic variation of coccoid bacteria, obtained variants by aging the cultures of the yellow pigment-producing coccus. Attempts were made to transform the cocci back to rods, but results were not successful. Rods would not develop, even on media upon which rods normally grow well. Koelz (12), in his work on Actinomyces, stated that the coccus form which developed from the rod form must be a stable form because it could not be transformed back to the rod condition.

Coccus forms appear on media which are not most favorable for growth and reproduction. In no case were definite rod forms obtained from the coccoid forms occurring in the finely granular and structureless lightly-staining material.

Increasing the concentration of lactose in the media transformed rods to coccus-like structureless material. Cultures grown in media in which growth was difficult also contained coccus-like forms instead of rods. Such media were: nutrient broth, nitrate peptone, egg albumin, dulcitol broth, Dunham's solution, rubber, hemp, lactose broth, and dextrose broth.

Rettger and Gillespie (15), in a study on cell morphology of *Bacillus megatherium*, found "relatively slight changes in environment are responsible for striking changes in cell form." They further state that "factors which stimulate cellular variation are apparently unfavorable to continued normal growth."

Transfers were made from lactose broth to the following media: nutrient agar, Czapek's agar, cornmeal agar, lactose broth, dulcitol broth, sucrose broth, 2% tryptose broth, salicin broth, dextrose broth, litmus milk, gelatin stab, nutrient broth, potato, cellulose, Loeffler's blood serum, citrate agar and starch agar. No growth occurred on: Czapek's cellulose, cornmeal and citrate agar. Starch was not hydrolized, neither nitrates, indol, acetyl methyl carbinol, nor acid (as indicated by methyl red) were produced. These results show that the coccus differs from the normal rod form in its reaction to culture media in the following ways: (1) starch is not hydrolized, while the rod form is actively diastatic. (2) Addition of starch to nutrient agar does not produce more favorable conditions for growth of the coccus form, while the rod form shows better growth when starch is added. (3) Good growth is obtained with the coccus form when incubated at 37° C. for 24 hours, while the rod form requires longer incubation. (4) The coccus form produces no pigment, while the rod form produces an orange pigment. Gillespie and Rettger (8), in their work on variant cells of *Bacillus megatherium*, consider the coccoid and rod forms as merely two extremes of cell length. Holman and Carson (10) question whether these changes in cell morphology are more than mere chance variation. The results in this work tend to agree with Gillespie and Rettger that unfavorable factors stimulate cellular variation. It is doubtful whether these are mere chance variations since repeated experiments on a large number of cultures show identical results.

18. Identification of the Bacterium

The characteristics of this organism may be summarized as follows: Filaments and rods: 0.8 to 1.2 by 2.4 to 60 microns. In older cultures mostly short rods. Frequently Y, swollen, and coccus forms, staining irregularly, showing granules. Non-motile. Gram-negative. Gelatin stab: orange surface growth. Very slow napiform liquefaction. Agar colonies: small, circular, smooth, convex, adherent to medium, compact, orange. Deep colonies lens-shaped. Agar slant: fair growth, filiform, smooth, light orange. Starch agar colonies: large, circular, smooth, moist, spreading, slimy, orange-pink. Starch agar slant: abundant, slimy, moist, spreading. Czapek's agar slant: fair growth, light pink, slightly slimy. Cornmeal agar slant: fair growth, light pink, slightly slimy. Nutrient broth: slight turbidity. Starch broth: moderate tur-

bidity, moderate sediment. LITMUS MILK: no change. POTATO: fair growth, coral-pink to vermillion-red, dry, lustreless. INDOL: not formed. NITRITES: not produced from nitrates. AMMONIA: not produced. ACID: from lactose. BLOOD SERUM: no growth. STARCH: hydrolyzed. HYDROGEN SULPHIDE: not formed. AEROBIC: facultative. OPTIMUM TEMPERATURE: 20-37° C. SOURCE: water in city distribution system. HABITAT: unknown.

Possibilities of identification. The following characters are similar to those of the genus Corynebacterium: uneven staining due to metachromatic granules, long slender rods, non-acid fast, pleomorphism, optimum growth under acid conditions, pigment production. Jensen (11), in his studies on saprophytic Mycobacteria and Corynebacteria describes a species which resembles this organism to some extent. *Corynebacterium michiganense* resembles this organism in the following respects: similar growth on potato, gelatin and broth; acid medium optimum, and scant growth at 37° C. The organism also shows some characteristics of *Corynebacterium nubium* which is feebly proteolytic and shows a pink growth on agar. Corynebacterium has the following characters which would exclude the present organism from inclusion in that genus: growth on paraffin, tendency for branching, angular growth ("snapping") Gram-positive, often club-shaped rods, growth on Loeffler's blood serum, optimum temperature 37° C.

The genus Actinomyces has the following characteristics which are similar to those of the present organism: pleomorphism, the organism segmenting into rods and coccoid forms, irregular staining showing "granules," pigment production, actively diastatic, not easily cultivated on artificial media, slow growth, no gas from carbohydrates, optimum temperature 13-32° C. Although no species of Actinomyces listed in Bergey's Manual (2) shows resemblance to this bacterium, the writer is impressed by many studies made on the genus which show similar results to those in the study of this organism. Novak and Henrici (14), in their work showing the relationship between staphylococci and actinomycetes, found that sugar was the inciting substance which caused morphological transformation. Koelz (12), in his work on 16 strains of Actinomyces, was unable to transform coccoid forms which developed from rod forms back to rod forms.

Actinomyces has the following characteristics which would exclude the present organism from inclusion in that genus: Gram-

positive, prefers alkaline medium, branching forms, clubbed ends of radiating threads, aerial outgrowths, mycelium, reproduction by conidia, growth usually dry, tough and wrinkled.

The genus Flavobacterium has the following characteristics which are similar to those of the present organism: Gram-negative rods, aerobic, orange pigment, occurs in water, feeble power of attacking carbohydrates. Many workers, studying bacteria found in chlorinated water supplies, place many of the chromogenic bacteria in this genus. Bender (1) assigned some of the organisms containing water-soluble pigments and which survive chlorination to the genus Flavobacterium. Levin (13) included members of this genus among the bacteria of water distribution systems. Charlton (4), in his work on chlorine-tolerant bacteria in water supplies, assigned most of the pigmented, Gram-negative rod-forms to Pseudomonas and Flavobacterium.

No species listed in Bergey's Manual (2) shows great similarity to the present organism. The following two species are similar in several ways to the present organism yet insufficiently so to permit it to be classified as either of them. *Flavobacterium orchitidis* has bipolar staining and is Gram-negative. *Flavobacterium aurantiacum* produces limited orange on agar slant, reddish-orange pigment on potato, is Gram-negative and has an optimum temperature of 30° C.

In view of these considerations it appears that this organism is a new species and it is proposed to assign to it the name *Flavobacterium amylum* sp. nov.

SUMMARY

1. A pigmented bacterium, capable of withstanding chlorination lethal to coliform organisms, has been isolated from the distribution system of a city water supply. It is easily recognized by the formation of an orange pigment at low incubation temperatures. It appears predominately during summer and early winter months.

2. Samples of raw water, plant water, plant effluent and taps (generally) do not show this pigmented organism.

3. The organism is difficult to grow on ordinary media, but grows and multiplies well on media containing soluble starch.

4. Cell morphology varies from long, filamentous rods through short rods to coccoid forms. The longer rods occur during growth

periods and under most favorable conditions. Shorter rods and cell variations appear after a longer incubation period and under conditions unfavorable to normal growth.

5. There is no indication of a morphological time cycle.

6. Granules found in the rod forms stain well with acetic methylene blue and give negative tests for sulphur and starch.

7. The organism, in the normal rod form, is actively diastatic reducing starch to glucose. As the concentration of starch in the medium is increased, better growth and longer rods are observed. In the coccoid form the organism does not hydrolyze starch and addition of starch to the medium does not induce more growth.

8. Better growth and longer rods are produced on media with small concentrations of lactose.

9. Media which are slightly acid produce larger amounts of growth but also shorter rods and more coccoid forms than are found on alkaline media.

10. The organism shows a slimy characteristic on certain media and this may partially account for its resistance to chlorine.

11. Optimum conditions for pigmentation are: incubation at 37° C. for 24 hours followed by re-incubation for 48 hours at 20° C. and on media of low pH values with concentrations of soluble starch up to 5%.

12. It may be assumed that the following conditions are unfavorable to growth of the organism: tempertaure higher than 37° C., dryness, early spring months, alkaline conditions and chlorination above lethal dosage for coliforms.

13. The new name, *Flavobacterium amylum* sp. nov. is assigned to the organism.

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